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13. ABSTRACT ***(Maximum 200 Words)***

Site-specific chromosome alterations have led to the identification of numerous genes directly involved in hematologic malignancies and selected solid tumors. Recognition of recurrent chromosome translocations are particularly informative because they can lead directly to the cloning and identification of genes which are altered in neoplasms. We hypothesize that, as been shown in other human tumors, early clinical breast cancers have site-specific, clonal chromosomal translocations which have not been identified previously. We are developing a new method, premature chromosome condensation (PCC),using mitotic *Xenopus* extracts that will allow us to obtain G-banded karyotypes from primary, uncultured breast cancer specimens. We have made such extracts and are optimizing conditions for use. We are also using a sensitive new technique termed spectral karyotyping (SKY), to identify site-specific, recurrent clonal chromosomal translocations in these PCC-induced karyotypes of primary breast cancer specimens. The problems in application at this point are technical and being addressed. Following the identification of recurrent, site specific chromosome alterations and particularly translocations in primary breast cancer, future investigations would target our long-term goal of identifying the genes directly involved at these chromosomal breakpoints.

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4. INTRODUCTION

We hypothesize that, as has been shown in other human tumors, early clinical breast cancers have site-specific, clonal chromosomal translocations and other abnormalities. Many such chromosomal breakpoints have not been identified yet because of selection of metaphases obtained in short term tissue culture and the frequent poor resolution obtainable with standard chromosome banding techniques. We expect to find site-specific chromosome translocations in breast cancer. The altered genes at some of these breakpoints will have a major role in the genesis or progression of breast cancer.

We are devising an innovative method to identify chromosome changes in individual cells of primary breast cancers. The successful application of this method, called premature chromosome condensation (PCC), for the study of breast cancer will allow us to directly describe clonal chromosome aberrations bypassing the short-term tissue culture methods currently required to obtain an adequate number of analyzable metaphases. By identifying structural clonal chromosome abnormalities in breast cancer specimens we will contribute to the ultimate development of accurate prognostic clinical tests. The proposed work may identify new genes directly involved in tumor initiation, metastasis, and/or drug resistance. These genes have direct relevance to breast cancer diagnosis and treatment.

5. BODY

Following are our progress associated with each task in the approved statement of work.

Technical objective 1. To develop a new method, premature chromosome condensation (PCC) that will allow us to obtain G-banded karyotypes from primary, uncultured breast cancer specimens; this method makes use of mitotic *Xenopus* extracts

Task 1. Obtain MCF 7 cell line, prepare nuclei to perform PCC (Month 1)

Cell lines obtained and screened for preliminary cytogenetics (baseline) analysis using traditional metaphase cytogenetics include:

DU4475	near tetraploid/ near triploid
T47D	hypotriploid
MCF-7	hypotetraploid

SK-BR-3 hypotetraploid
Hs578T near-tetraploid/near-pentaploid
We decided to prepare several different cell lines, as the ploidy of the cell lines used may influence the success of the PCC approach applied. Nuclei have been prepared and frozen.

Task 2. Obtain Xenopus extract needed for PCC (Month 1)

We made 3 different preparations of extract, under the direction of Dr Kathy Wilson, modifying the preparation method from published protocols described in our original proposal. We tested the extracts on nuclei from both a normal diploid lymphoblast line and from the MCF 7 breast cancer cell line. While some chromosome condensation was seen, it was not adequate to obtain the data we are seeking. Addition of recombinant cyclin B kinase just prior to initiating the PCC procedure greatly improved the efficiency of the extracts.

This took about six months, due to time required to prepare frogs, seasonal variation in egg quality from frogs, and time required to test extracts.

Task 3. Optimize PCC with MCF7 or other breast cancer cell line (Months 1-3)

We next sought to increase the number of interphase nuclei showing PCC. A number of factors were addressed:
1) density of nuclei on slides
2) adequate adherance of nuclei to slides, including comparison of cyto-spin technique with traditional methods of slide making
3) determination of best hypotonic solution to use, which would not interfere with the PCC reaction. Five different hyptonic solutions were tried. We encountered some difficulty with crystallized residue from proteins in the extract reacting with fixative.

This has taken longer than the 3 months estimated in our proposal, and optimization is still in progress. A significant publication appeared several months ago which has influenced our approach to PCC (Prasanna et al, 2000). Briefly, this article describes the use of okadaic acid, a phosphatase inhibitor and $p34^{cdc2}$/cyclin B kinase to induce PCC in the absence of fusion with mitotic cells or Xenopus extract. We are in the process of trying to replicate this work. If successful, modification of this technique for application to breast cancer could accelerate our ability to study the chromosomal aberrations we

seek. We are also trying addition of okadaic acid to the existing xenopus extracts to see if extract efficiency can be improved in inducing PCC.

Examples of some of the PCC we have obtained using Xenopus extract are shown in Figure 1.

Task 4. Collect and process nuclei from primary breast cancer specimens (Months 1-28)

We are in the process of collecting nuclei from primary breast cancer specimens. Before freezing many specimens, we are comparing the efficiency of induction of PCC from nuclei processed as described in older literature vs. alternate methods. This is a key factor in the successful induction of PCC.

Task 5. Optimize PCC on primary breast cancer specimens (Months 4-7)

We are saving our primary breast cancer specimens until we have optimized the method on the cell line material.

Task 6. Write manuscript reporting application of method (Months 8-10)

This will occur when the method is optimized.

Technical objective 2. To apply a sensitive new technique, multi-plex fluorescent in situ hybridization, termed spectral karyotyping (SKY), to identify site-specific, recurrent clonal chromosomal translocations and other consistent chromosomal alterations in PCC-induced karyotypes of primary breast cancer specimens.

Task 1. Collect nuclei from primary breast cancer specimens (Months 1-28)

We are collecting primary breast cancer specimens, as noted above.

Task 2. Perform PCC, obtain G-banded karyotypes from primary breast cancer specimens (Months 9-30)

We will not perform PCC on primary breast cancer specimens until the methodology has been optimized.

Task 3. Perform spectral karyotyping on primary breast cancer specimens (Months 9-30)

While our PCC methodology is not yet optimized, several recent publications confirm that spectral karyotyping is a useful technique for identification of chromosomal rearrangements in primary breast tumors (Adeyinka et al, 2000; Kytola S et al, 2000). We have been evaluating the effect of various factors and PCC conditions on our ability to obtain good quality spectral karyotyping (SKY) images. We have encountered significant problems with autofluorescence of chromosomes, and have just traced this to the DAPI counterstain and eliminated the problem. Examples of our current state of SKY data obtained from PCC-processed nuclei from a lymphoblast cell line are shown in Figure 2.

Task 4. Analyze breakpoints and genomic abnormalities on each specimen (Months 9-30) :

This will be performed when PCC is optimized and applied.

Task 5. Summarize data on ideogram (Months 9-33):

This requires completion of Task 4.

Task 6. Write final report, manuscript, plan and write proposals for identifying genes at the specific selected breakpoints found in this project (Months 34-36) :

This will occur when the method is optimized and applied.

Technical objective 3. For each breakpoint found in tumors from two or more patients, we propose to localize and define the breakpoint using region-specific FISH probes.

Task 1. Obtain and label region-specific probes (Months 14-30, depends on accumulation and location of breakpoints)

Task 2. Perform spectral karyotyping and/or routine FISH with region specific probes on relevant primary tumors (Months 16-32)

Task 3. Summarize data on ideogram (Months 16-32)

Task 4. Write final report, manuscript, plan and write proposals for identifying genes at the specific selected breakpoints found in this project (Months 34-36)

None of these tasks were within the first year of proposed work.

6. KEY RESEARCH ACCOMPLISHMENTS

- Xenopus extract which can induce PCC has been made
- Optimization of PCC extract is underway
- Collection of primary breast cancer nuclei for analysis is underway
- Optimization of nuclear preparation is underway
- Evaluation of technical problems encountered when combining PCC and SKY is underway.

7. REPORTABLE OUTCOMES

At this point, we do not have any of the reportable outcomes listed in the instructions for preparing this report (manuscripts, abstracts, presentations; patents/licenses; degress/ development of cell lines; informatics; other funding applied for).

8. CONCLUSIONS

We are off to a good start in this high-risk, but potentially high-yield avenue of research seeking to develop a novel method to identify consistent chromosomal translocations in human breast cancer primary patient specimens.

9. REFERENCES

1. Adeyinka A, Kytola s, Mertens F, Panidis N, Larsson C. Spectral karyotyping and chromosome banding studies of primary breast carcinomas and their lymph node metastases. Int J Molec Med 5: 235-240, 2000.

2. Kytola S, Rummukainen J, Nordgren A, Karhu R, Farnebo F, Isola J, Larsson C Chromosomal alterations in 15 breast cancer cell lines by comparative genomic hybridization and spectral karyotyping. Genes Chromosomes Cancer 28:308-17, 2000.

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FIGURE LEGENDS

Figure 1.

Panels A-C show examples of cells in which premature chromosome condensation (PCC) has been induced using mitotic Xenopus extract. Chromosomes have been solid-stained with Leishman's stain.

Figure 2.

Two examples of cells in which premature chromosome condensation (PCC) has been induced using mitotic Xenopus extract, stained by spectral karyotyping (SKY).

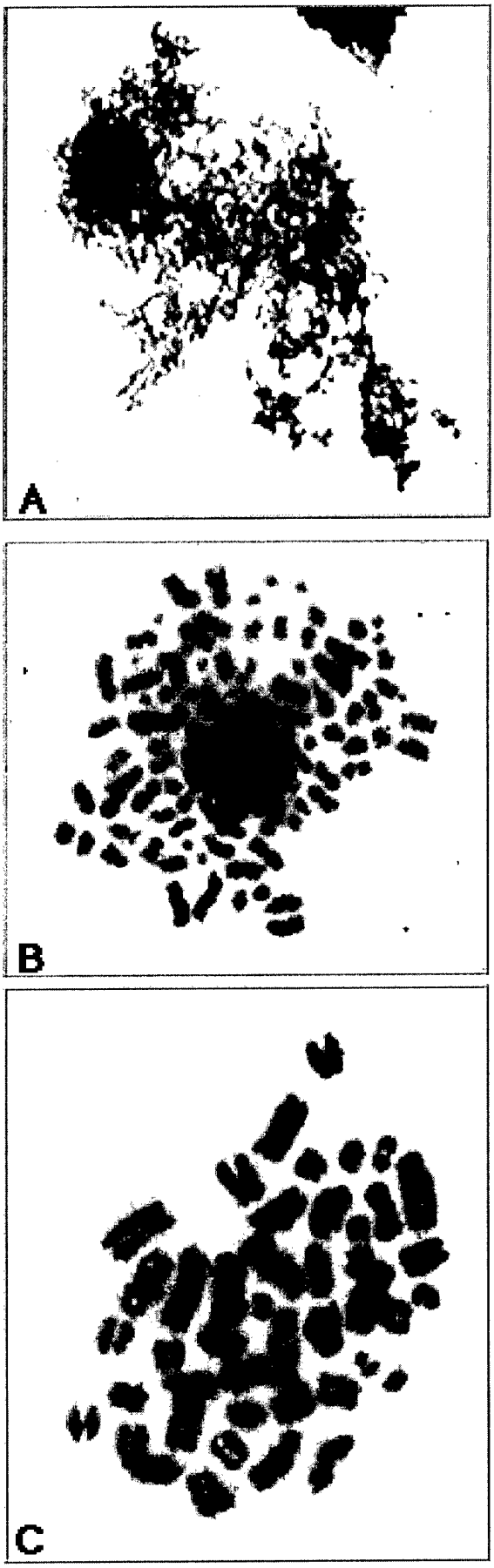

Figure 1

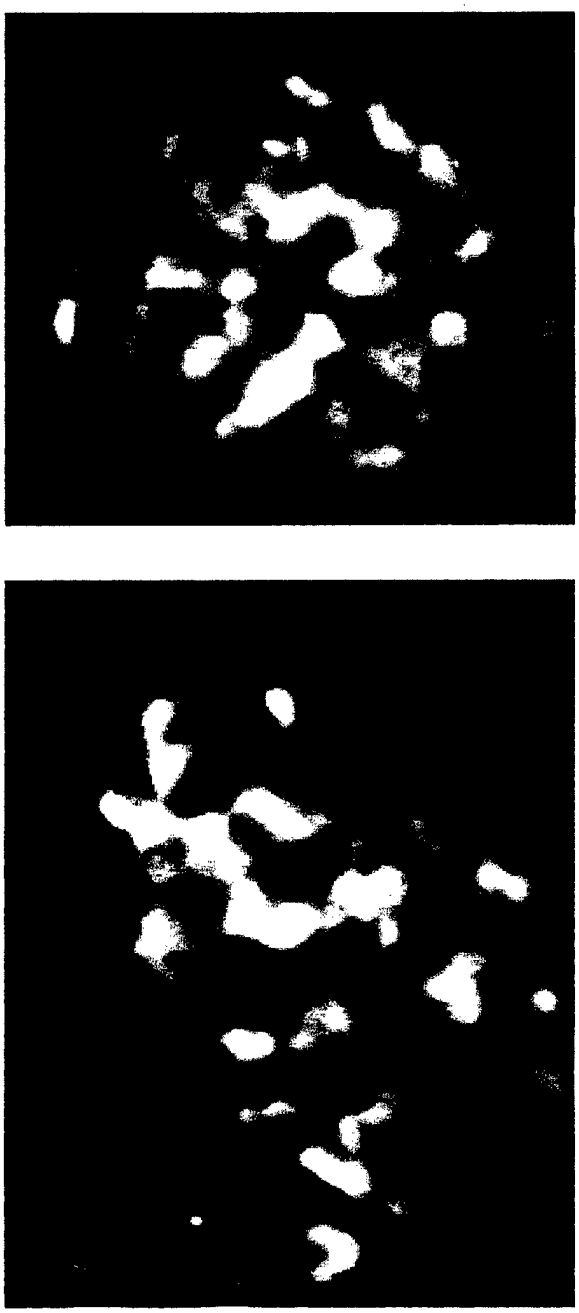

Figure 2